ALLOZYME VARIATION AND TAXONOMY OF THE RIVER BLACKFISH, *GADOPSIS MARMORATUS* RICHARDSON, IN WESTERN VICTORIA

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A taxonomic study of the river blackfish, *Gadopsis marmoratus* was conducted using allozyme and morphological data. Focussing upon populations from western Victoria and south-eastern South Australia, a total of 147 blackfish from 14 locations were scored for variation at 28 allozyme loci. The occurrence of fixed allelic differences between samples less than 50 kilometres apart suggests that *G. marmoratus* comprises distinct northern and southern forms, consistent with previous allozyme studies. While the degree of allozyme differentiation between the northern and southern forms is small for freshwater fish species, their recognition as distinct species is justified on the basis of the limited geographic allozyme variation within each form and the finding of a geographically abrupt genetic discontinuity in western Victoria. An examination of dorsal fin spine counts also indicated differences between northern and southern forms. However, a high degree of variation within the northern form of *G. marmoratus* reduces the value of this trait for diagnostic purposes, not only for distinguishing between the two genetic forms of *G. marmoratus*, but also between *G. marmoratus* and *G. bispinosus*.

Key words: Blackfish, *Gadopsis marmoratus*, allozyme electrophoresis, morphology, taxonomy, Australia.

THE FAMILY Percichthyidae represents a distinctive element of Australia's freshwater fish fauna. Placed within this family are the morphologically distinctive gadopsid fish which are endemic to south-eastern Australia and carry out their entire life cycle in freshwater. There is conjecture on the evolutionary affinities of this taxon as it is thought that the genus *Gadopsis* has either evolved from a marine ancestor some 15 million years ago, or had a more ancient Gondwanan freshwater origin (Sanger 1984). If the latter is true the family should command a high conservation status, similar to that of the Australian lungfish (*Neoceratodus forsteri*) and the two species of Saratoga (*Scleropages* spp.) (Grant 1997).

The genus *Gadopsis* contains just two currently recognised species, *Gadopsis marmoratus* (Richardson 1848) and *G. bispinosus* (Sanger 1984). *Gadopsis marmoratus*, commonly known as the river blackfish, was described by Richardson in 1848 and was believed to be the sole gadopsid species. Some 136 years after Richardson's original description, Sanger (1984) described a second species, *Gadopsis bispinosus* the two spined blackfish, based upon morphological and allozyme data. The two species have contrasting distributions. *Gadopsis bispinosus* has a restricted distribution in the upper reaches of the Murray River, whereas *G. marmoratus* has a very wide distribution throughout the Murray-Darling River system, southern and easterly draining river systems in Victoria, and also in northerly flowing rivers of Tasmania. The species has also been introduced to southern Tasmania (Jackson et al. 1996).

The two *Gadopsis* species have been taxonomically distinguished on the basis of dorsal spine number, with *G. bispinosus* having one to three, and *G. marmoratus* having 6-13. The morphological differences between the two species are supported by allozyme data, which indicate genetic differences in sympatry (Sanger 1986).

There is, however, a degree of ambiguity regarding the taxonomy of *G. marmoratus*. McCoy (1879) described a third species, *Gadopsis gracilis* from the southern flowing Yarra River, distinguishable on the basis of head dimensions. However, Ogilby (1913) considered this to be an inconsistent trait and suggested *G. gracilis* to be a junior synonym of *G. marmoratus*. Parrish (1966) suggested *Gadopsis* from northern Tasmania to be a distinct species, however, this notion was dismissed by Sanger (1984) who considered the Tasmanian fish to be conspecific with

mainland populations. Sanger (1986) did however find allozyme variation between *G. marmoratus* populations north and south of the Great Dividing Range and he referred to these as the northern and southern forms of *G. marmoratus*.

A difficulty with the recognition of the two forms as discrete species, as pointed out by Sanger (1986), is that the degree of allozymic divergence between them is less than normally found between distinct species, and that their reproductive status cannot be determined as the two species have not been found in sympatry (Richardson et al. 1986). Sanger (1986) recommended that further taxonomic studies of the *G. marmoratus* complex be undertaken with special emphasis upon sampling in south-eastern South Australia and south-western Victoria, where he considered it likely that northern and southern forms occur sympatrically. It has also been suggested that populations of *Gadopsis* in the south-east corner of South Australia may be genetically distinct, especially those of the Ewens Ponds area, due to the species' relatively fragmented distribution in this state (Jackson et al. 1996).

The objective of this study was to extend the genetic and taxonomic research of Sanger (1986) on *G. marmoratus* via comprehensive sampling in south-western Victoria and south-eastern South Australia. The specific objectives were to: (1) determine whether sympatric populations of northern and southern *G. marmoratus* coexist in the Glenelg, Wimmera or coastal drainages of south-western Victoria and south-eastern South Australia, as suggested by Sanger (1986); (2) evaluate the taxonomic status and genetic relationships of *G. marmoratus* samples from these regions using allozyme electrophoresis; and (3) to evaluate variation in dorsal spine counts, the principal trait used to taxonomically distinguish *Gadopsis* species.

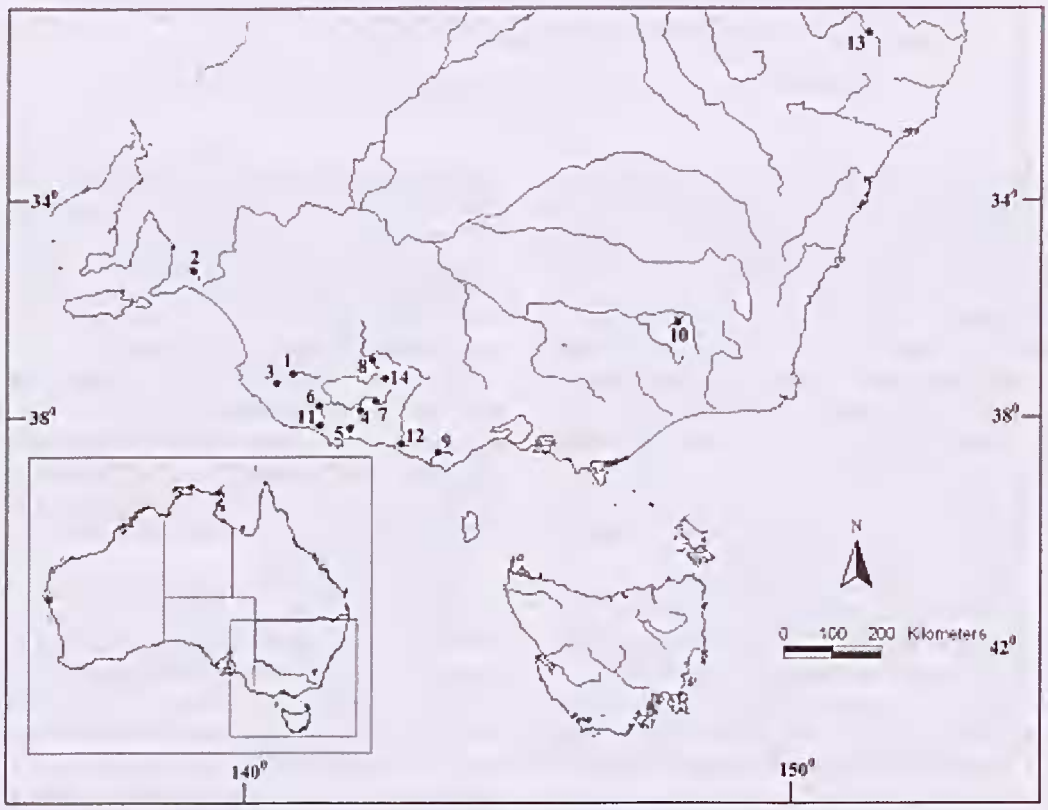

Fig. 1. Sample locations: 1. Mosquito Creek, 2. Angas River, 3. Eight Mile Creek, 4. Muddy Creek, 5. Darlot Creek, 6. Glengallan Creek, 7. Wannon River, 8. McKenzie River, 9. Gellibrand River, 10. Cudgewa Creek, 11. Glenelg River, 12. Brucknell Creek, 13. MacDonald River, 14. Scrubby Creek.

MATERIALS AND METHODS

Collection of samples

Gadopsis samples were obtained between February and September 1999 from 14 sites, three from South Australia, one from New South Wales and 10 from Victoria. Samples were collected via hook and line, bait trap, and electrofishing techniques. Four of the 14 sites were selected as reference sites based upon previous studies (Sanger 1984; Ovenden et al. 1988), including two sites from the Murray/Darling catchment for northern *G. marmoratus* (MacDonald River and Angas River), one site from a south-west Victorian coastal drainage as a reference site for southern *G. marmoratus*, (Gellibrand River), and one site in north-eastern Victoria (Cudgewa Creek) representing *G. bispinosus*.

Details of sample sites and the number of fish sampled are summarised in 'Fig. 1' and 'Table 1'. All specimens were transported back to the laboratory on ice, where they were stored at –80°C. Subsequently, liver and muscle samples were dissected from each specimen and placed into plastic vials and stored at –80°C until needed for electrophoresis. All carcasses were preserved in ethanol for later reference.

Electrophoresis

Electrophoresis was carried out using standard techniques (Farrington et al. 1999). Buffer volumes and running conditions followed standard procedures (Murphy et al. 1990) and staining solutions were prepared using standard recipes (Shaw & Prasad 1970; Harris & Hopkins 1976). All individuals were scored for variation at 28 allozyme loci. The enzyme systems used, the number of loci scored for each enzyme together with enzyme abbreviations, Enzyme Commission (EC) numbers and details of running conditions and buffers used are given in 'Table 2'. Loci were designated by abbreviations of the enzyme stains used. In the case of multiple loci for a given enzyme, they are labeled numerically starting at the locus coding for the most anodal isozyme. Cathodally migrating allozymes are designated with a minus sign. Allozymes were designated by relative mobility with the most common allozyme labelled 1.00 (Table 3).

Site	River/Creek	Species/Form[1]	Location	N
1	Mosquito Creek	*G. marmoratus* - N	S.A. (Narracorte)	13
2	Angas River	*G. marmoratus* - N	S.A. (Strathalbyn)	4
3	Eight Mile Creek	*G. marmoratus* - N	S.A.(Ewens Ponds)	6
4	Muddy Creek	*G. marmoratus* - N	Vic (Hamilton)	15
5	Darlot Creek	*G. marmoratus* - S	Vic (Heywood)	13
6	Glengallan Creek	*G. marmoratus* - N	Vic (Dartmoor)	18
7	Wannon River	*G. marmoratus* - N	Vic (Grampians)	22
8	McKenzie River	*G. marmoratus* - N	Vic (Wartook)	22
9	Gellibrand River	*G. marmoratus* - S	Vic (Gellibrand)	17
10	Cudgewa Creek	*G. bispinosus*	Vic (Cudgewa)	5
11	Glenelg River	*G. marmoratus* - N	Vic (Harrow)	4
12	Brucknell Creek	*G. marmoratus* - S	Vic (Brucknell)	1
13	MacDonald River	*G. marmoratus* - N	N.S.W. (Armidale)	2
14	Scrubby Creek	*G. marmoratus* - N	Vic (Grampians)	5

[1] N = northern form, S = southern form

Table 1. Sample sites, including site number, abbreviated names, species or form, location of sample site and number of individuals sampled (N).

Enzyme	E. C. Number	Abbreviation	Tissue	No. of loci	Buffer system
Adenosine deaminase	3.5.4.4	ADA	L	1	TEB
Alcohol dehydrogenase	1.1.1.1	ADH	L	1	TEB
Arginine Kinase	2.7.3.3	AK	L	1	LIOH
Creatin Kinase	2.7.3.2	CK	L	1	LIOH
Esterase	3.1.1.1	EST	L	1	TEB
Fumarate Hydratase	4.2.1.2	FUMH	L	1	LIOH
General protein	———	GPT	M	3	TEB
Glycyl-L-leucine	3.4.11.	GL	L	1	TEB
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PD	L	1	TC-6
Glutamate dehydrogenase	1.4.1.3	GDH	L	1	TEB
Glutamate-oxaloacetate transaminase	2.6.1.1	GOT	L	1	PLK
Isocitrate dehydrogenase	1.1.1.42	IDH	M	1	TEB
Lactate dehydrogenase	1.1.1.27	LDH	L	2	TEB
Malate dehydrogenase	1.1.1.37	MDH	M	1	LIOH
Malic enzyme	1.1.1.40	ME	L	1	TC-6
Mannose-6-phosphate isomerase	5.3.1.8	MPI	L	1	TC-6
Nucleoside phosphorylase	2.4.2.1	NP	L	1	TEB
L-Leucyl-glycl-glycine peptidase	3.4.11.4	LGG	L	1	TEB
L-Leucyl-proline peptidase	3.4.13.9	LP	L	1	PLK
L-Leucyl-tyrosine peptidase	3.4.13.11	LT	L	1	TEB
Phosphoglucomutase	2.7.5.1	PGM	L	1	LIOH
Phophoglucose isomerase	5.3.1.9	PGI	M	1	TEB
Sorbitol dehydrogenase	1.1.1.14	SDH	L	1	PLK
Superoxide dismutase	1.15.1.1	SOD	L	1	TEB

Table 2. Enzymes, abbreviations, Enzyme Commission (E.C.) numbers, tissue type, number of loci and buffer sustems used in the electrophoretic study of the Gadopsidae. Tissue type: L, Liver; M, Muscle. Buffer systems: TEB, Tris-EDTA-borate buffer No.6 (Selander et al. 1971); LtOtl, LiOtl-Boric Acid buffer No.2 (Selander et al. 1971); TC-6, Tris-citrate buffer No.4 (Selander et al. 1971); PLK, Poulik electrode buffer (Ballment et al. 1993).

Statistical analyses

Comparisons among samples were made using Nei's genetic identity, calculated from allelic frequencies, which were summarised by UPGMA clustering. All calculations were performed using the Tools for Population Genetics Analysis software paekage (Miller 1997).

Morphological Analysis

Variation in dorsal spine counts has been the principal diagnostic trait used to distinguish *G. marmoratus* from *G. bispinosus* (Sanger 1984). Counts were made by eye except for small specimens for which counts were determined under a dissecting microscope.

RESULTS

A total of 147 blackfish from 14 sites were scored for variation at 28 presumptive gene loci. Eighteen loci showed no variation: ADA, AK, CK, EST, FUMH, G6PD, GDH, GPT-2, GPT-3, IDH, MDH, ME, MPI, NP, LDH-2, LP, PGM, and SDH. Nine loci showed variation between sites in the form of fixed allelic differences (Table 3). Fish from Cudgewa Creek, representing *G. bispinosus*, showed the greatest divergence with fixed allelic differences at 5 of 28 loci (18 %) compared to all other samples. Fish from Darlot Creek, Brueknell Creek and the Gellibrand River were homozygous for an alternate allele at 3 out of 28 loci (equivalent to 11% fixed allelic differences) compared to all other samples excluding Cudgewa Creek (*G. bispinosus*). Fish from Darlot Creek were ho-

Locus	Allele	Site 1 Mosquito (13)	2 Angas (4)	3 Eight Mile (6)	4 Muddy (15)	6 Glengallan (18)	7 Wannon (22)	8 McKenzie (22)	11 Glenelg (4)	13 MacDonald (2)	14 Scrubby (5)	5 Darlot (13)	9 Gellibrand (17)	12 Brucknell (1)	10 Cudgewa (5)
ADH	-100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00
	-25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00
GPT-1	112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
GL	114	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
GOT	111	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00
LDH-1	-100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00
	-36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00
LGG	106	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00
	90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00
LT	109	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
GPI	100	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00
	92	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
SOD	112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00	1.00	1.00

Table 3. Allele frequencies at each locus for all 14 sites of *Gadopsis* spp. Sample sizes in brackets below site codes. Loci are designated by abbreviation of the enzyme names, with multiple loci labelled numerically beginning with the locus coding for the most anodal isozyme. Cathodally migrating alleles are designated by a minus sign. Alleles are scored for relative mobility to the most common allele at each locus which is labelled 100.

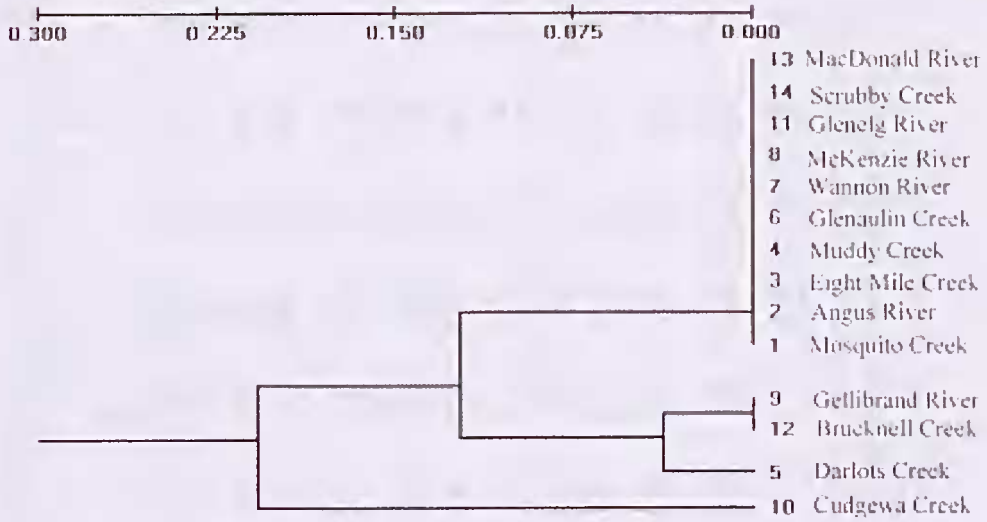

Fig. 2. UPGMA dendorgram summarizing genetic relationships among river blackfish samples derived from a matrix of Nei's unbiased (1978) Genetic Distance.

mozygous for a single unique allele at the GPI locus. Intra-sample variation was extremely limited, consisting of a single heterozygote at each of the GPI and SOD loci from the entire sample of 147 fish.

Genetic relationships among sample sites are summarised in a UPGMA dendrogram ('Fig. 2') derived from Nei's identity (1978). This analysis reveals three distinct clusters. The Mosquito, Eight Mile, Muddy, Glengallan, and Scrubby creeks and the Angas, Wannon, McKenzie, Glenelg, and MacDonald rivers form a single distinct group that clusters with a second comprising Darlot Creek, the Gellibrand River, and Brueknell Creek, with the most distinct sample, Cudgewa Creek, representing *G. bispinosus*. The former two clusters represent northern and southern *G. marmoratus*. Within the southern *G. marmoratus* cluster, Darlot Creek clusters apart from the other two samples on the basis of a single fixed difference at the GPI locus. Quite remarkably, with the exception of a low frequency allele at GPI in the Muddy Creek sample, no variation was found among the samples of northern *G. marmoratus* despite these samples having been obtained from 4 major river systems (Glenelg, Wimmera, Murray and Darling rivers) and from isolated water bodies in south-eastern South Australia across a range of some 1,200 km.

Spine counts could not be obtained for all fish sampled due to damage to some specimens. 'Table 4' shows that the dorsal spine counts for *G. bispinosus* individuals was either 1 or 2, whereas the spine counts for *G. marmoratus* were highly variable, ranging from 3 to 13. Variation within samples was generally limited and conspicuous differences between populations were apparent. For example, spine counts in the Darlot Creek sample ranged from 11 to 13, whereas in the Wannon River sample they ranged from 3 to 6. The southern form of *G. marmoratus* (Brueknell Creek, Gellibrand River, Darlot Creek) had relatively high counts (9 to 13) whereas the northern form had generally lower counts (3 to 11).

DISCUSSION

Sanger (1986) reported allozyme differentiation between several populations of *G. marmoratus* from northern and southern Victoria. The results of this study extend the findings of Sanger (1986) in several respects. It has significantly increased the geographic range of sampling of *Gadopsis* for genetic and taxonomic analysis and also the number of allozyme loci examined. More specifically, it confirms that significant allozyme differences occur between northern and southern forms of *G. marmoratus*. However, no evidence was found for the sympatric occurrence of the northern and southern forms of blackfish as speculated by Sanger (1986). The Fitzroy River / Darlot Creek system appears to be the most

Site	Species/Form[1]	N	Dorsal Spine Count	
			mean	range
Mosquito Creek	*G. marmoratus* - N	13	9.69	9 - 11
Angas River	*G. marmoratus* - N	4	10.50	10 - 11
Eight Mile Creek	*G. marmoratus* - N	6	9.00	8 - 10
Muddy Creek	*G. marmoratus* - N	15	8.13	7 - 9
Darlot Creek	*G. marmoratus* - S	13	12.46	11 - 13
Glengallan Creek	*G. marmoratus* - N	18	8.33	8 - 10
Wannon River	*G. marmoratus* - N	19	4.80	3 - 6
McKenzie River	*G. marmoratus* - N	17	9.18	8 - 10
Gellibrand River	*G. marmoratus* - S	15	11.33	9 - 13
Cudgewa Creek	*G. bispinosus*	5	1.60	1 - 2
Glenelg River	*G. marmoratus* - N	4	7.75	7 - 8
Brucknell Creek	*G. marmoratus* - S	1	13.00	13
MacDonald River	*G. marmoratus* - N	2	9.00	9
Scrubby Creek	*G. marmoratus* - N	5	6.80	6 - 8

[1] N = northern form, S = southern form

Table 4. Dorsal spine counts for blackfish from 14 sites in south-eastern Australia

westerly limit of the southern form of *G. marmoratus* with the northern form appearing to be relatively common and widespread in western Victoria. In this study northern *G. marmoratus* was found at several sites in the Glenelg and Wannon river systems, the headwaters of the Wimmera River and also in the small drainages of south-eastern South Australia. The results of this study also indicate that there is no evidence for more than one taxonomic form of *Gadopsis* in South Australia (Jackson & Llewellyn 1980).

The failure to find the northern and southern forms of *G. marmoratus* in sympatry prevents the taxonomic status of the forms from being evaluated on the basis of whether or not they are reproductively isolated. In contrast *G. bispinosus* shows five fixed differences (18.5% fixed differences) in comparison with *G. marmoratus* samples. As these genetic differences are maintained in sympatry, it can also be concluded that these taxa represent valid biological species (Sanger 1984). The determination of the taxonomic status of allopatric populations in general, and for freshwater fish in particular, is a persistent problem (McDowall 1972). However, the pattern of geographic variation in allozyme divergence within *G. marmoratus* supports the existence of two biological species.

Specifically, a striking feature of the allozyme data is the lack of genetic differentiation between samples in unconnected drainages, some 1200 km apart (ie: Wannon and MacDonald River samples). This contrasts with the four fixed allozyme differences found between populations of northern and southern *G. marmoratus* from Glengallan and Darlot Creek respectively, less than 50 km apart. These results suggest that there has been a connection between the Murray River and the rivers of south-west Victoria in the relatively recent past, and that a barrier, either biological (competitive exclusion) or geological, has existed between blackfish populations inhabiting the southerly flowing drainages in south-west Victoria for a significant period of time.

Additional support for the possibility that the northern and southern forms represent distinct species comes from an examination of evolutionary relationships of *Gadopsis* species by Ovenden et al. (1988) using restriction analysis of mitochondrial DNA. Although only based upon limited sampling of northern *G. marmoratus*, these authors found that the level of divergence between the northern and southern forms of *G. marmoratus* was of the same order of magnitude as between *G. bispinosus* and *G. marmoratus*. Further, their analyses, in contrast to the allozyme data, indicated that the relationship among the three forms were equidistant and formed an unresolved trichotomy. We are currently examining nucleotide sequence variation in the mitochondrial 12S rRNA gene region between the samples of

Gadopsis collected in this study in a effort to throw more light on the taxonomy and phylogeography of the river blackfish especially in the western parts of its distribution.

The level of intra-population variation within blackfish in this study is low and similar to the findings of Sanger (1986). The absence of allozyme variation within populations of freshwater fish species and other freshwater organisms is, however, not unusual (Campbell et al. 1994; Avery & Austin 1998; Jerry et al. 1999). The low levels of genetic diversity within populations of freshwater organisms is usually attributed to low or fluctuating population sizes and the discontinuous nature of freshwater environments. Under these circumstances genetic variation will be lost due to stochastic processes (genetic drift and founder effects) and the introduction of new genes into populations will be uncommon as gene flow will be rare between isolated water bodies.

Sanger (1986) used dorsal spine number to distinguish *G. bispinosus* and *G. marmoratus*. According to Sanger (1986), *G. bispinosus* exhibits a dorsal spine range of 1 to 3 and *G. marmoratus* a range of 6 to 13. New dorsal spine count data from this study indicates that this character is more variable than previously realised in *G. marmoratus*, with individuals from the Wannon River having counts ranging down to 3. Thus while this trait remains an important taxonomic characteristic, it cannot be considered a completely reliable diagnostic feature for distinguishing between *G. bispinosus* and *G. marmoratus*. It is also noteworthy that while northern and southern forms of *G. marmoratus* show overlap in dorsal spine counts, the northern form shows consistently lower counts compared with the southern form and therefore provides some independent support for the allozyme data.

In conclusion, although this study failed to find sympatric populations of 'northern' and 'southern' forms of *G. marmoratus*, it did discover abrupt genetic discontinuity between them in western Victoria. This supports their recognition as discrete species. We recommend that a decision on the appropriate names for the species identified in this study be held in abeyance pending a thorough taxonomic review. A major issue affecting the nomenclature of blackfish is uncertainty regarding the type locality for *G. marmoratus*, which may have been within the range of either northern or southern forms (Richardson, 1848, Ogilby, 1913).

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